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Influence of pH, Dissolved Oxygen, Suspended Solids or Dissolved Solids Upon Ventilatory and Cough Frequencies in the Bluegill 'Lepomis macrochirus' and Brook Trout 'Salvelinus fontinalis'

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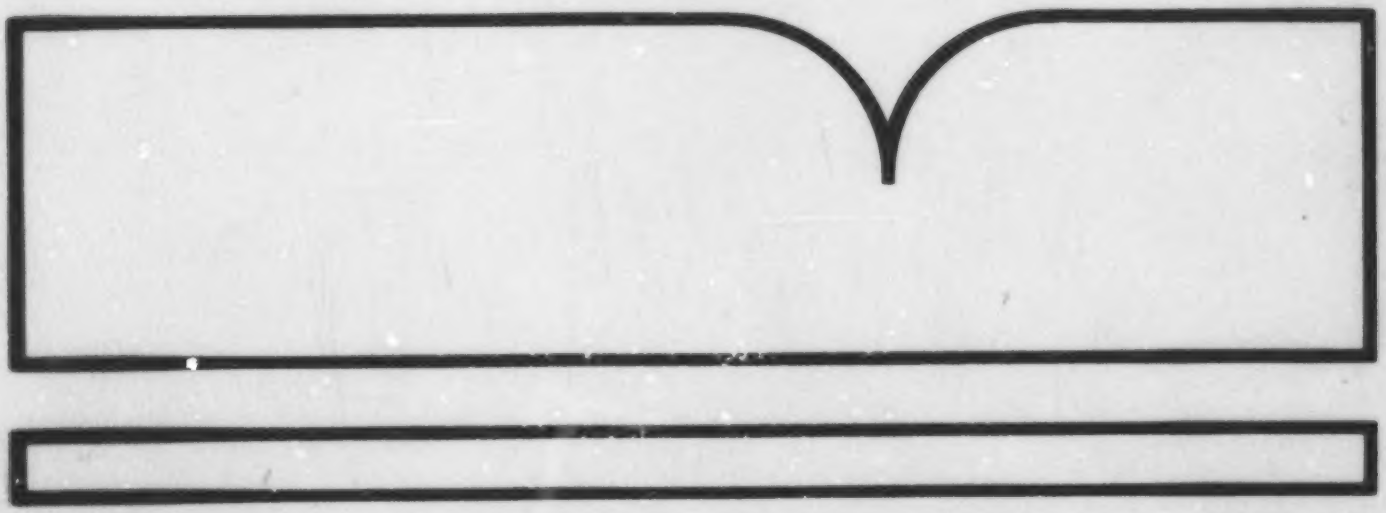

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16. ABSTRACT

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Bluegill ventilatory and coughing responses were slightly more sensitive than those of brook trout. Bluegills showed increased ventilatory frequency in dissolved oxygen concentrations below 77% air saturation, pH 6.7 and below, and hardness of 190 mg litre^{-1} (as $CaCO_3$) or higher, but no increase in ventilation in suspended solids concentrations up to 323 NTU of clay particles. Brook trout responsed with increased ventilation only in concentrations of DO at 44%, pH of 6.0 and 231 NTU suspended solids. Ventilatory frequency responses at low pH were attributed to increased free CO_2 released by acidification of the dilution water.

Over the DO range tested (44-117%) coughing was unaffected in both species. Bluegill coughing increased at pH levels above 9.0, but not below the control pH of 8.0. Increases also occurred in some suspended solids concentrations (90 NTU and above) and in hardness of 190 mg litre^{-1} and above. Brook trout coughing was unaffected over the pH range 5.1 - 10.1 and at all suspended solids (0.6 - 318 NTU) concentrations tested. The only coughing response observed for brook trout in different dissolved solids concentrations occurred at 1022 mg litre^{-1} hardness.

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The Influence of pH, Dissolved Oxygen, Suspended Solids or Dissolved Solids Upon Ventilatory and Cough Frequencies in the Bluegill *Lepomis macrochirus* and Brook Trout *Salvelinus fontinalis*

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ABSTRACT

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Bluegill ventilatory and coughing responses were slightly more sensitive than those of brook trout. Bluegills showed increased ventilatory frequency in dissolved oxygen concentrations below 77% air saturation, pH 6·7 and below, and hardness of 190 mg litre^{-1} (as $CaCO_3$) or higher, but no increase in ventilation in suspended solids concentrations up to 323 NTU of clay particles. Brook trout responded with increased ventilation only in concentrations of DO at 44%, pH of 6·0 and 231 NTU suspended solids. Ventilatory frequency responses at low pH were attributed to increased free CO_2 released by acidification of the dilution water.

Over the DO range tested (44–117%) coughing was unaffected in both species. Bluegill coughing increased at pH levels above 9·0, but not below the control pH of 8·0. Increases also occurred in some suspended solids concentrations (90 NTU and above) and in hardness of 190 mg litre^{-1} and above. Brook trout coughing was unaffected over the pH range 5·1–10·1 and at all suspended solids (0·6–318 NTU) concentrations tested. The only coughing response observed for brook trout in different dissolved solids concentrations occurred at 1022 mg litre^{-1} hardness.

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INTRODUCTION

Coughing and ventilatory responses are sensitive, short-term indicators of adverse effects in fish exposed to single aquatic contaminants at sublethal levels (Morgan, 1976; Drummond & Carlson, 1977). Additionally, fish will act as biological sensors when exposed to complex effluents, presumably presenting a summarised response to the numerous possible toxicants and antagonistic substances that may be present in a particular effluent. For these reasons, biomonitoring tests have been developed that use changes in ventilatory frequency to warn of deleterious changes in potable water (Morgan, 1978) or developing toxic conditions in an industrial effluent (Cairns & Gruber, 1979). Also, changes in cough frequency have been used to screen toxic complex effluents from those that would probably exert little or no effect on aquatic life (Carlson & Drummond, 1978).

Detection limits for these tests vary but are quite consistent considering the variety of toxic substances and number of fish species tested so far. Sloof (1979) assessed rainbow trout *Salmo gairdneri* as a biological indicator and found the limits for detection lay between 1% (cadmium) and 30% (acrylonitrile, cyanide, lindane) of the 48-h LC_{50} values for zebrafish *Brachydanio rerio*. However, Morgan (1976) showed that sensitivity varied between fish species and concluded that the response limits lay between 5 and 10% of the 48-h LC_{50}. So far as is known, coughing response limits are equally sensitive. Davis (1973) exposed sockeye salmon *Oncorhynchus nerka* to bleach kraft mill effluent and found increased coughing at 20% of the 96-h LC_{50}, and water-soluble crude oil fractions caused pink salmon fry *Oncorhynchus gorbuscha* cough frequency to increase at 30% of the 96-h LC_{50} (Rice *et al.*, 1977). Drummond *et al.* (1973) detected coughing responses near the maximum acceptable toxicant concentration of 9–17 $\mu g\,litre^{-1}$ established for brook trout *Salvelinus fontinalis* exposed to copper.

This degree of sensitivity, coupled with the fact that fish cannot identify just what it is they are responding to in experimental waters, poses special problems for biomonitoring systems based on changes in respiratory functions. For example, some complex effluents are known measurably to alter, singularly or in combination, the pH, dissolved oxygen (DO), suspended solids and dissolved solids levels of the receiving waters into which they are discharged. Within concentration ranges that are quite wide, these physical variables are considered non-toxic, but little is known

on how fish respiratory functions react to deviation from levels in these variables normally encountered by the fish (Cairns & van der Schalie, 1980). If fish acting as sensors of potentially toxic conditions respond to minor deviations caused by effluent discharge, even though the alteration remains within non-toxic limits, then the biomonitoring test will issue a false positive result. This failure to distinguish between toxic components in the effluent and harmless changes in environmental quality is a problem and, clearly, responses to these deviations must be accounted for if ventilation and coughing are to be reliable indicators of effluent toxicity.

The objective of this research was to document the effects that altered pH, or changes in DO, suspended solids and dissolved solids concentrations had on coughing and ventilatory frequencies of bluegill sunfish and brook trout. These species were chosen because both have been used in coughing and ventilation studies at this laboratory and elsewhere, and the bluegill has been used in an established biomonitoring system (Cairns & Gruber, 1979).

Besides those selected here, there are other non-toxic environmental variables that may also affect the results of ventilatory biomonitoring systems. Temperature is certainly important and has been shown to affect ventilatory frequency during biomonitoring (Cairns *et al.*, 1974); it may affect coughing as well (Walden *et al.*, 1970). Photoperiodism affects diurnal cycling of ventilatory frequency (Sparks *et al.*, 1972; Price, 1979), and there is some evidence that circadian rhythms play a rôle in ventilatory responses (Dively *et al.*, 1977). Vibration, whether due to seismic shock (Sparks & Cairns, 1972) or something else, will cause fish to alter their ventilatory patterns. However, the four aforementioned variables were considered the most critical because they are ever-present in biomonitoring situations, because they are subject to sudden change beyond immediate control and because little is known about how these changes affect ventilatory responses.

MATERIALS AND METHODS

Test apparatus

The test system included electrode chambers similar to those described by Drummond & Carlson (1977). Each chamber was constructed of glass and was divided into four separate and watertight compartments. Baffles

separated each 13 cm long × 3·5 cm wide × 10 cm high fish compartment from the water inlet and drain. A stainless steel wire electrode was mounted on each baffle such that incoming or outflowing water did not impinge directly on the electrode. Also, the electrodes were shielded with short sections of Tygon tubing mounted a few millimetres apart to prevent interference problems caused by the fish touching the bare electrodes. Each electrode pair was connected via patch cords to a junction box and from there shielded two-conductor cables led through a switching device to high gain capacitor-coupled preamplifiers set at a time constant of 0·3. After passing through channel amplifiers, the signals were recorded on a rectilinear strip-chart recorder.

Two electrode chambers were placed in each of four 'viewing chambers'. This chamber was a 55-litre all-glass aquarium with one-way glass glued to one side and the remaining three sides shielded with styrofoam. Changes in ventilating or coughing patterns because of the fish's reaction to an observer were thus avoided. A wooden cover had holes drilled in it for the necessary water supply lines, grounding wires, patch cords and sampling lines. An incandescent aquarium light with two 25 W bulbs rested over a slot in the cover and provided a moderate level of light within the chamber. The daylight–darkness simulator of Drummond & Dawson (1970) turned on or off the fluorescent room lights just before it gradually brightened or dimmed the lights on each viewing chamber. The photoperiod was 16 h light:8 h dark, and dark shower curtains suspended around the test apparatus reduced background illumination during daylight hours.

Constant head distribution boxes placed on each cover received acclimation water or test solution from mixing cells and delivered it down glass tubes to each fish compartment. Flow rate to each compartment was set at approximately 45 ml min^{-1} with glass capillary tubes and, once set, seldom varied by more than 2 or 3 ml during a test. Except for dissolved oxygen tests, the various stock solutions were fed from their containers to the mixing cells with a peristaltic pump. Each mixing cell contained a stirring bar and rested on a small magnetic stirrer.

Biological and chemical methods

Lake Superior water used for holding and acclimation of test fish, as well as dilution water for test concentrations, was charcoal-filtered and aerated in reservoirs to maintain dissolved oxygen (DO) close to air

saturation. Test temperature was $11{\cdot}0 \pm 1{\cdot}8$ °C for brook trout and $21{\cdot}5 \pm 1{\cdot}2$ °C for bluegills. During a test, daily measurements were made of temperature, DO, pH, acidity, alkalinity and hardness in at least one experimental concentration. Continuous flow sample lines were run from each distribution box to a site a few feet from the testing area so that sampling would not disturb the fish. All concentrations of the test variable were measured twice daily. Suspended solids of control water were determined only during that test series, and conductivity was measured during the pH and dissolved solids test series. Total CO_2 of Lake Superior control water was determined using a modified Van Slyke volumetric blood–gas analyser, and free CO_2 in differing pH test waters, as well as control water, was calculated from a table of proportions for CO_2, HCO_3^- and $CO_3^=$ in water at various pH values (Hutchinson, 1957). Except for free CO_2, all chemical analyses were made according to standard methods (American Public Health Association, 1976).

A metering box provided dissolved oxygen concentrations between 40 and 100% air saturation. The box was partitioned into two equal compartments and charcoal-filtered water from a vacuum degasser flowed through one constant level compartment while gas-saturated water flowed through the other. Tapping each compartment with glass capillary tubes produced a 350 ml min^{-1} flow of degassed and saturated water into each of eight mixing cells located directly below the metering box. Selecting the right combination of capillary tubes with differing internal diameters yielded the proper degassed to saturated water ratios for desired DO test concentrations. Slightly supersaturated gas concentrations used in some tests were obtained directly from the heated incoming lake water supply line.

Test water pH was altered by the addition of hydrochloric acid (HCl) or sodium hydroxide (NaOH) to distilled water in stock bottles and the stock solution was then pumped to the appropriate mixing cell. Desired test concentrations were obtained before fish were placed in the system by monitoring the pH in the electrode chambers and adding 1 N solutions to stock bottles as required. Once the pH range was set, the test solution flows were turned off until the start of exposure and the electrode chambers were flushed with dilution water to restore normal pH.

Stock solutions of suspended solids were prepared with red clay collected from the south shore of Lake Superior near Port Wing, WI. Five baseball-sized clumps of clay were added to 19 litres of distilled water and the container placed on a laboratory shaker for 10 min. The resulting

suspension was allowed to sit undisturbed for 24 h while the larger particles and debris settled out. This was necessary to obtain particles small enough to pass through the flow metering capillary tubes. The supernatant was siphoned off, stored at 4 °C, and the procedure repeated until sufficient stock was accumulated to last one test run. Just before testing, the supernatant was added to a 150-litre all-glass headbox where a submersible pump and aeration maintained the particles in suspension.

Spoor (1977) measured Ca as 14 mg litre^{-1} and Mg as 3 mg litre^{-1} in Lake Superior water. Other constituents included Na (1·1 mg litre^{-1}), K (0·5 mg litre^{-1}), Cl (1·2 mg litre^{-1}), SO_4 (3·2 mg litre^{-1}) and HCO_3 (40 mg litre^{-1}). Because magnesium and calcium contributed the most to hardness, typically 42–45 mg litre^{-1} (as $CaCO_3$) in Lake Superior water, magnesium sulphate ($MgSO_4 . 7H_2O$) and calcium chloride ($CaCl_2$) were used to provide stock solutions for the dissolved solids tests. Separate stocks of these were prepared and fed to mixing cells in the same 14 Ca:3 Mg ratio.

Juvenile bluegill sunfish obtained from the Lake Mills National Fish Hatchery were reared in large holding tanks at the Environmental Research Laboratory-Duluth (ERL-D). They averaged 106 mm (range 84–138) in total length and 16·9 g (range 6·2–41·2) in weight. Juvenile brook trout, raised from stock hatched at ERL-D, averaged 123 mm (range 95–144) in total length and 14·9 g (range 11·1–22·8) in weight. Both species were fed frozen brine shrimp and pelletised trout food *ad libitum*. Fish were not fed during tests.

Test procedures

Bluegills transferred to electrode chambers were allowed to acclimate 3 days, and brook trout 4 days, before monitoring began (Drummond & Carlson, 1977). Recording of background cough and ventilatory frequencies began at the end of acclimation and the first 24 h was designated as Day 0. Recording was continuous throughout Day 0 and for four subsequent test days. Each group of four fish represented one concentration and were monitored for 15 min during each hour before switching to the next group. During a single exposure, either three concentrations and a control group or seven concentrations and a control were tested. There were no duplicates. Additional test runs replicated most test concentrations or added new concentrations to the test series. At the end of testing, the fish were killed, weighed and measured.

A representative 5-min segment from each monitoring period for each fish was scanned for coughs and the frequency recorded as coughs per 5 min. A 1-min ventilation count was similarly obtained. The tabulated data were entered on a computer file and the biomedical computer (BMDP) statistical program P5D (Chasen, 1979) was used to calculate daily means, along with standard deviations, for each group of four fish over the 5-day test period. Dunnett's procedure (one-way) (Steel & Torrie, 1960) for comparison of treatment means with a control was used to determine whether or not a group of fish showed significant increases in coughing or ventilatory frequencies during each day of the test when compared with their respective background frequencies (Day 0). One group of fish in each test was a designated test control. These fish received only Lake Superior water throughout the test period and their ventilation characteristics were checked to ensure that any differences found in exposed fish were not due to an altered incoming water supply.

RESULTS

Chemistry

Chemical characteristics of Lake Superior water used for holding, acclimation and test control fish, as well as experimental water, are given in Table 1. For all controls combined, Lake Superior water had a mean pH of 8·0, a total hardness (as $CaCO_3$) of 43 mg litre^{-1}, an average alkalinity (as $CaCO_3$) of 39 mg litre^{-1}, acidity (as $CaCO_3$) of 1·3 mg litre^{-1} and a mean conductivity of 93 μmhos cm^{-1} at 25°C. Except for experimental concentrations in the dissolved oxygen tests, DO remained above 90% air saturation. Total CO_2 of Lake Superior water was measured six times and averaged 29·0 mg litre^{-1}. Calculated free CO_2 was 0·7 mg litre^{-1} at pH 8·0.

Dissolved oxygen

Bluegill sunfish were sensitive to DO concentrations below 77% air saturation, but not consistently so (Table 2). Some groups showed significant increases in ventilatory frequency at 72, 63, 60, 55 and 46% while replicates in some of these same concentrations and at 68, 66, 60, 54 and 51% saturation showed increasing ventilation but not so much as to

TABLE 1
Means for Chemical Characteristics of Lake Superior Water (Controls) and Experimental Water

Test series	*Range of mean concentrations*	*pH*	*Dissolved oxygen*	*Hardness*	*Alkalinity*	*Acidity*	*Conductivity*	*Calculated free CO_2*
	pH		*Per cent saturation*	*mg litre^{-1} as $CaCO_3$*	*mg litre^{-1} as $CaCO_3$*	*mg litre^{-1} as $CaCO_3$*	*μmhos cm^{-1} at 25°C*	*mg litre^{-1}*
	9·8–10·1		96	44	64	NM	138	0·006
	8·5–9·2		95	41	44	NM	102	0·02
	7·8–8·2[a]		97	43	40	1·5	96	0·7
pH	6·6–7·5		96	44	30	6·1	103	5·5[b]
	5·9–6·1		96	44	12	17·3	115	20·3
	5·0–5·3		95	44	4	23·3	125	27·8
	4·2–4·3		97	46	NM	NM	NM	28·6
	Per cent saturation							
	106–117	7·8		42	38	1·9	NM	0·7
	92–102[a]	8·0		42	37	1·3	NM	0·7
	82–85	7·9		42	38	1·4	NM	0·7
Dissolved	75–79	8·0		42	39	1·5	NM	0·7
oxygen	66–72	8·0		42	37	1·1	NM	0·7

	60–63	8·0		42	38	1·7	NM	0·7
	51–55	8·0		43	38	1·3	NM	0·7
	44–46	8·0		43	37	1·6	NM	0·7
	mg litre^{-1} *as* $CaCO_3$							
Dissolved	916–1 022	7·7	95		41	2·8	1 867	0·7
solids	406–473	7·8	92		41	1·9	954	0·7
(hardness)	190–205	7·9	92		40	1·4	443	0·7
	42–44[a]	7·9	94		40	1·0	91	0·7
	NTU[c]							
	311–318	8·4	94	62	83	NM	NM	0·2
Suspended	231–276	8·3	91	54	52	NM	NM	0·2
solids	122–197	8·3	91	52	42	NM	NM	0·2
	76–94	8·3	94	48	46	NM	NM	0·2
	0·4–0·9[a]	7·9	92	40	35	1·2	NM	0·7

[a] Lake Superior water controls.
[b] Free CO_2 present at pH 7·0.
[c] Nephelometric turbidity units.
NM Not measured.

TABLE 2

Ventilatory Responses of Bluegill Sunfish *Lepoomis macrochirus* Exposed to Various Concentrations of Non-Toxic Environmental Variables

Dissolved oxygen					*pH*					*Suspended solids*				
% saturation	*VF/min*[a]		*CF/5-min*[b]		*VF/min*			*CF/5-min*		*NTU*	*VF/min*		*CF/5-min*	
	Day 0	*Maximum*[c]	*Day 0*	*Maximum*	*pH*	*Day 0*	*Maximum*	*Day 0*	*Maximum*		*Day 0*	*Maximum*	*Day 0*	*Maximum*
117	21 ± 6[d]	21 ± 8	6·5 ± 1·8	12·6 ± 7·6[f]	10·1	29 ± 9	28 ± 7	6·8 ± 2·4	29·2 ± 24·3	323	20 ± 4	22 ± 5	5·4 ± 1·3	8·3 ± 2·2
114	29 ± 8	49 ± 33[e]	8·4 ± 2·6	14·5 ± 12·7	10·1	24 ± 6	24 ± 8	6·7 ± 1·5	15·2 ± 7·8[g]	312	21 ± 4	20 ± 8	4·7 ± 1·8	6·7 ± 2·0
					10·0	29 ± 6	29 ± 17	6·3 ± 2·0	9·7 ± 5·4					
101[h]	28 ± 11	31 ± 11	8·5 ± 2·5	10·0 ± 3·2						272	22 ± 3	23 ± 6	4·2 ± 1·3	5·8 ± 1·9
99[g]	28 ± 10	33 ± 15	6·1 ± 2·1	6·3 ± 2·1	9·8	18 ± 5	20 ± 6	5·4 ± 1·2	9·3 ± 4·1	253	20 ± 4	23 ± 8	4·2 ± 1·3	8·2 ± 2·6[g]
					9·8	31 ± 7	27 ± 15	5·3 ± 1·7	24·4 ± 18·5[g]	232	20 ± 4	20 ± 6	4·6 ± 1·4	7·2 ± 2·7
96[i]	29 ± 7	29 ± 7	5·9 ± 1·9	6·7 ± 2·0										
92[i]	21 ± 7	33 ± 12	7·9 ± 2·4	8·0 ± 3·0	9·1	28 ± 5	24 ± 6	5·5 ± 1·6	7·5 ± 2·6	197	23 ± 5	23 ± 7	4·3 ± 1·5	7·0 ± 2·3
					9·1	23 ± 5	32 ± 14	6·6 ± 2·3	15·4 ± 13·3	122	22 ± 4	28 ± 4	4·8 ± 1·2	8·2 ± 2·2[g]
85	29 ± 7	41 ± 11	6·3 ± 2·3	6·3 ± 1·7										
					8·7	26 ± 4	28 ± 10	6·1 ± 1·3	6·5 ± 1·9	90	21 ± 7	20 ± 6	3·7 ± 1·2	5·8 ± 1·4[g]
82	27 ± 8	30 ± 10	6·5 ± 2·5	6·8 ± 1·9	8·5	24 ± 6	23 ± 7	6·2 ± 1·6	8·1 ± 2·9	76	20 ± 4	20 ± 5	3·5 ± 1·0	4·6 ± 1·5
82	25 ± 6	28 ± 8	9·1 ± 2·9	14·7 ± 15·8										
					8·2[i]	24 ± 4	23 ± 4	6·3 ± 2·7	7·1 ± 2·9	0·9[i]	19 ± 3	19 ± 3	3·6 ± 1·1	4·1 ± 1·2
79	25 ± 6	31 ± 7	8·2 ± 2·4	9·7 ± 3·6	8·1[i]	31 ± 7	30 ± 8	5·8 ± 1·9	7·7 ± 3·9	0·4[i]	21 ± 4	22 ± 3	4·3 ± 1·4	4·4 ± 1·2
77	27 ± 11	34 ± 10	7·5 ± 2·4	7·4 ± 2·7						0·4[i]	20 ± 3	23 ± 5	3·3 ± 1·1	4·3 ± 1·8
					8·0[i]	24 ± 7	24 ± 9	6·4 ± 1·3	5·7 ± 1·4					
72	19 ± 3	27 ± 5[g]	6·9 ± 1·7	8·0 ± 2·8	8·0[i]	17 ± 5	21 ± 6	4·4 ± 1·3	7·3 ± 4·2					
72	26 ± 6	34 ± 13	8·7 ± 3·2	11·6 ± 4·7[e]										
72	25 ± 4	40 ± 16[g]	7·3 ± 2·5	8·2 ± 3·3	7·5	28 ± 8	28 ± 8	5·7 ± 1·7	6·5 ± 2·6					
					7·3	23 ± 4	23 ± 4	5·9 ± 1·5	5·9 ± 1·6					
69	29 ± 12	42 ± 17	6·4 ± 3·0	15·1 ± 10·6										
68	23 ± 6	35 ± 9	6·1 ± 2·2	10·2 ± 10·6	6·9	31 ± 7	38 ± 9	5·6 ± 1·6	8·2 ± 2·2					
					6·8	21 ± 6	23 ± 5	6·6 ± 1·5	5·7 ± 1·2					
66	20 ± 6	28 ± 10	7·3 ± 3·1	8·3 ± 4·4	6·7	21 ± 5	35 ± 14[g]	5·6 ± 1·9	8·6 ± 3·9					
63	26 ± 6	54 ± 15[h]	8·3 ± 1·9	9·8 ± 3·1	6·3	17 ± 3	31 ± 11[g]	4·2 ± 1·0	6·7 ± 2·6					
60	25 ± 6	40 ± 10[g]	7·1 ± 2·7	9·6 ± 2·7	6·0	22 ± 5	50 ± 16[h]	6·2 ± 2·3	8·7 ± 4·4					
60	32 ± 8	52 ± 20	7·4 ± 2·2	10·8 ± 5·0	6·0	27 ± 6	59 ± 15[h]	6·4 ± 2·0	7·9 ± 2·6					
					6·0	24 ± 7	36 ± 12	6·6 ± 1·3	6·3 ± 2·3					
55	25 ± 10	42 ± 10[g]	7·0 ± 2·3	10·0 ± 2·9										
54	20 ± 6	32 ± 12	6·1 ± 2·1	9·1 ± 3·8	5·3	15 ± 4	33 ± 14[g]	4·2 ± 1·1	5·7 ± 1·6					
					5·3	26 ± 7	51 ± 14[h]	6·5 ± 1·9	8·2 ± 2·5					
51	30 ± 8	51 ± 23	8·5 ± 2·9	9·7 ± 3·9										
46	20 ± 5	36 ± 10[g]	7·7 ± 3·1	11·2 ± 6·2	5·1	20 ± 3	51 ± 12[h]	6·4 ± 1·7	7·2 ± 5·0					
					4·3	21 ± 3	54 ± 18[h]	6·8 ± 2·1	13·5 ± 3·7					

Dissolved solids				
mg litre^{-1} (*as* $CaCO_3$)	*VF/min*		*CF/5-min*	
	Day 0	*Maximum*	*Day 0*	*Maximum*
929	21 ± 4	45 ± 17[g]	6·0 ± 1·3	9·8 ± 5·7
916	20 ± 5	25 ± 7	2·8 ± 1·3	6·7 ± 1·2[h]
459	21 ± 4	34 ± 15	5·5 ± 1·3	8·2 ± 1·5
406	18 ± 3	22 ± 11	3·6 ± 1·7	5·3 ± 2·8
197	20 ± 4	21 ± 5	3·9 ± 1·7	5·1 ± 2·1
190	20 ± 3	34 ± 10[g]	4·4 ± 1·4	7·9 ± 2·6[g]
44[i]	18 ± 4	18 ± 3	3·2 ± 1·5	3·5 ± 1·0
44[i]	20 ± 3	21 ± 4	5·4 ± 1·5	6·3 ± 1·9

[a] VF = ventilatory frequency; [b] CF = cough frequency; [c] maximum = maximum mean frequency (one of four exposure days); [d] mean ± standard deviation, $n = 4$ fish/concentration except where indicated—means based on 24 counts (less in some cases) per fish per day; [e] $n = 3$; [f] $n = 1$; [g] $p = 0{\cdot}95$ Dunnett's procedure (1-sided) was used to compare each day of exposure (treatment) with Day 0 (control); [h] $p = 0{\cdot}99$; [i] test controls.

be significant ($p = 0{\cdot}95$). At 46%, significant increases persisted over the first 3 days of exposure whereas increased ventilation lasted only 1 or 2 days at higher concentrations and generally on exposure Days 3 and 4. Gas supersaturated water (117%) killed all bluegills by the end of 3 days and one fish died in 114%. Except for increased, but non-significant, ventilatory frequency on Days 3 and 4 at 114%, there was no indication that anything was wrong with the fish held in gas supersaturated water.

Brook trout ventilatory frequency responses, or lack thereof, were more consistent (Table 3). Decreasing DO levels had little effect until 44% saturation, and they remained unaffected in slightly supersaturated water (106%).

Over the DO range tested, no effect was evident on coughing in either species.

pH

Bluegill sunfish had consistently higher ventilatory frequencies at low pH (6·7–4·3) compared with those at, or near, control pH, and maintained near normal frequencies at high pH (8·5–10·1). The reverse pattern was true for bluegill coughing; increased coughing occurred at pH 9·8 and above while coughing remained close to normal at all pH values down to 5·1 and only slightly elevated at pH 4·3 (Table 2).

Except for control fish at pH 7·8, brook trout tests in waters of differing pH were not replicated. Brook trout ventilatory frequency increased at lower pH values of 6·0 and 5·2, but not significantly at the latter (Table 3). There was no effect on ventilation at pH values higher than control pH, and coughing remained unaffected over the range tested (pH 4·3–10·1).

Response times were quick for both species; increased ventilation or coughing occurred during Day 1 of exposure and at extreme pH the responses persisted over the test period.

Suspended solids

Other than a 1-day increase in ventilatory frequency for brook trout held in 231 nephelometric turbidity units (NTU) suspended clay particles, there were no discernible effects on ventilation in either brook trout or bluegills at concentrations as high as 323 NTU. Bluegill cough frequency increased in all test groups but neither significantly so for all replicates nor at the highest concentrations (312 and 323 NTU). Significant effects

TABLE 3

Ventilatory Responses of Brook Trout *Salvelinus fontinalis* Exposed to Various Concentrations of Non-Toxic Environmental Variables

Dissolved oxygen					*pH*					*Suspended solids*				
% saturation	*VF/min*[a]		*CF/5-min*[b]		*pH*	*VF/min*		*CF/5-min*		*NTU*	*VF/min*		*CF/5-min*	
	Day 0	*Maximum*[c]	*Day 0*	*Maximum*		*Day 0*	*Maximum*	*Day 0*	*Maximum*		*Day 0*	*Maximum*	*Day 0*	*Maximum*
106	43 ± 9[d]	44 ± 9	4·6 ± 1·3	5·2 ± 1·6	10·1	32 ± 5	33 ± 12	2·6 ± 0·8	4·0 ± 1·1	318	37 ± 6	51 ± 16	3·4 ± ·9	4·4 ± 1·9
102	46 ± 8	43 ± 4	5·4 ± 2·0	5·6 ± 1·9						311	31 ± 3	36 ± 10	3·8 ± 1·1	4·2 ± 1·2
					9·1	31 ± 5	30 ± 6	3·6 ± 1·6	2·8 ± 0·9					
100[f]	54 ± 11	53 ± 9	4·9 ± 1·4	5·0 ± 1·5						276	33 ± 6	36 ± 7	3·0 ± 1·0	3·3 ± 1·0
99[f]	45 ± 5	44 ± 5	5·0 ± 1·4	6·1 ± 1·6	8·6	33 ± 7	34 ± 8	3·2 ± 1·0	2·8 ± 0·8	231	37 ± 3	52 ± 16[g]	4·1 ± 1·2	4·2 ± 1·7
83	42 ± 13	43 ± 13	4·6 ± 1·5	5·6 ± 1·6	7·8[f]	37 ± 6	36 ± 6	2·9 ± 1·2	2·9 ± 1·1	146	32 ± 3	36 ± 6	3·1 ± 1·1	3·5 ± 1·4
82	44 ± 7	46 ± 8	5·0 ± 1·6	5·4 ± 1·3	7·8[f]	34 ± 7	34 ± 6	3·1 ± 1·0	3·0 ± 1·0	94	37 ± 8	42 ± 12	4·9 ± 1·2	5·4 ± 1·6
82	40 ± 7	41 ± 7	4·5 ± 1·6	6·2 ± 1·3										
					6·8	30 ± 6	33 ± 6	3·4 ± 1·1	3·7 ± 1·2	0·7[h]	38 ± 5	37 ± 5	4·9 ± 1·0	4·7 ± 0·9
78	42 ± 8	45 ± 7	5·5 ± 1·6	6·9 ± 2·3						0·6[f]	36 ± 7	37 ± 6	4·2 ± 1·0	5·1 ± 1·3
76	49 ± 17	51 ± 16	4·4 ± 1·3	5·4 ± 1·3	6·0	34 ± 4	44 ± 9[g]	3·2 ± 0·9	3·6 ± 1·6					
75	43 ± 5	48 ± 9	4·5 ± 1·4	6·2 ± 1·8										
					5·2	36 ± 4	44 ± 9	3·8 ± 1·3	4·0 ± 1·1					
61	44 ± 3	52 ± 8	5·4 ± 1·6	8·0 ± 2·2										
60	43 ± 7	49 ± 9	5·5 ± 1·7	7·6 ± 2·1										
52	41 ± 6	47 ± 8	5·6 ± 2·0	7·7 ± 2·7										
52	42 ± 5	52 ± 9	6·2 ± 1·4	8·5 ± 2·0										
46	44 ± 8	52 ± 9	4·8 ± 1·8	6·6 ± 2·1										
44	45 ± 7	63 ± 11[e]	5·3 ± 1·5	7·6 ± 1·7										

Dissolved solids				
mg litre^{-1} (as $CaCO_3$)	*VF/min*		*CF/5-min*	
	Day 0	*Maximum*	*Day 0*	*Maximum*
1 022	39 ± 8	54 ± 17	3·1 ± 0·9	5·4 ± 1·0[e,h]
1 001	39 ± 4	45 ± 15	3·6 ± 1·5	5·4 ± 2·0[f]
473	37 ± 7	53 ± 17	2·8 ± 1·0	3·6 ± 1·1
465	33 ± 5	46 ± 12[e]	3·4 ± 1·4	5·0 ± 1·8[e]
205	37 ± 4	45 ± 6	3·8 ± 0·9	5·0 ± 1·2
201	32 ± 4	39 ± 7	4·5 ± 2·9	4·1 ± 1·6
43[f]	31 ± 3	31 ± 3	2·4 ± 1·0	3·6 ± 2·5
42[f]	34 ± 4	32 ± 3	2·8 ± 0·8	3·3 ± 0·9

[a] VF = ventilatory frequency; [b] CF = cough frequency; [c] maximum = maximum mean frequency (one of four test days); [d] mean ± standard deviation, $n = 4$ fish/concentration—means are based on 24 counts (less in some cases) per fish per day; [e] $n = 2$; [f] $n = 1$; [g] $p = 0{\cdot}95$ Dunnett's procedure (1-sided) was used to compare each day of exposure (treatment) with Day 0 (control); [h] $p = 0{\cdot}99$; [i] test controls.

occurred at 90, 122 and 253 NTU on the first day of bluegill exposure (Table 2). In all cases responses were maximum during exposure Day 1. Brook trout cough frequency (Table 3) was unaffected throughout their exposure range (94–318 NTU).

Dissolved solids

Bluegills showed sporadically increased ventilation and coughing when exposed to dissolved solids in the hardness range of 44–929 mg litre^{-1} (as $CaCO_3$) (Table 2). Ventilation increased significantly in 190 and 929 mg litre^{-1}, while coughing was higher at 190 and 916 mg litre^{-1}. Brook trout responded with increased coughing only at 1022 mg litre^{-1}; coughing and ventilation were unaffected at all other dissolved solids levels (Table 3). In those groups that did respond, ventilation increased on Day 3 or Day 4 of testing whereas cough increases occurred during Day 1 and persisted throughout exposure.

DISCUSSION

No-effect concentration ranges were estimated for ventilatory and coughing responses of bluegill sunfish and brook trout exposed to changes in concentration of non-toxic environmental variables (Table 4). They represent the ranges within which these water quality characteristics can vary without causing a false alarm to be issued by applied

TABLE 4

Estimated No-Effect Ranges for Ventilatory and Coughing Frequency Responses of Bluegill Sunfish *Lepomis macrochirus* and Brook Trout *Salvelinus fontinalis* Exposed to Non-Toxic Environmental Variables in Lake Superior Water

	Bluegill sunfish		*Brook trout*	
	Ventilation	*Cough*	*Ventilation*	*Cough*
Dissolved oxygen	75–100[a]	50–100	60–100	45–100
pH	7·0–10·0	5·0–9·0	7·0–10·0	5·0–10·0
Suspended solids	0–>323[b]	0–80	0–200	0–>325
Dissolved solids	<40–200[c]	<40–200	<40–500	<40–500

[a] Per cent air saturation.

[b] Concentration of clay particles expressed as nephelometric turbidity units (NTU).

[c] mg litre^{-1} hardness as $CaCO_3$ due to addition of Mg and Ca.

biomonitoring systems using these species and Lake Superior or similarly soft water as a dilution water source. These are purposely conservative limits and were set such that any concentration where ventilation and coughing increased (Tables 2 and 3), whether significant at the 95% level or not, would lie outside the limits.

The limits were almost the same for both species, but the bluegill was slightly more sensitive to changes in these variables than brook trout. Response to lowered DO was one example. Typically, salmonids respond to decreased dissolved oxygen with an increased depth of ventilation without a concomitant increase in ventilatory frequency whereas the converse is true for the bluegill (Marvin & Heath, 1968; Hughes & Saunders, 1970). Because change in ventilatory amplitude was difficult to measure accurately with non-contact electrodes (Carlson, 1982), it was not measured in the tests conducted here, but brook trout and bluegill sunfish reacted similarly in ventilatory frequency. The bluegill began to show some sensitivity to lower DO at concentrations around 70%, while brook trout did not ventilate significantly faster until the DO dropped below 50%.

Increased ventilatory frequencies for both species upon exposure to lowered pH was probably due to increased free CO_2 in the water, not to the hydrogen ion concentration. Although Lake Superior water is soft, acidification of the test water resulted in free CO_2 concentrations rising from a calculated value of 0·7 mg litre^{-1} at a control pH of 8·0 to 28 mg litre^{-1} at pH 4·2 (Table 1). Transient increases in ventilatory frequency began to occur at pH 6·7 for the bluegill and pH 6·0 for brook trout, and became more persistent at the lower concentrations studied for the bluegill. Pegg & Jenkins (1976) showed that ventilatory frequency was highly variable but frequently increased by three times the standard rate for bluegills in acid water of pH 4·0–3·0, and Dively *et al.* (1977) observed 25–65% increases in opercular movements of brook trout at pH 4·2 compared with controls at pH 7·1. However, in neither case was the CO_2 concentration mentioned. Presumably, CO_2 was not considered a problem because recirculating, well-aerated waters were used in one study and a proportional diluter delivered well-aerated test solutions to aquaria in the other. Nevertheless, Neville (1979*a*) pointed out that it is essential to specify the CO_2 concentration, as well as the pH, of ambient water used to acidify exposures; he showed that fish remained calm and without increased ventilation once aerated water had driven off excess CO_2 in recirculating static tests. Furthermore, Neville (1979*b*) compared short-

term (45 min) and long-term (5 days) effects on rainbow trout ventilation due to an abrupt pH change from 7·0 to 4·0 in both hard and decarbonated waters. Significant increases occurred in the short-term hard water exposure but not in the decarbonated water; long-term fish were calm and without any consistent increase in ventilatory frequency. Hoglund & Hardig (1969) also concluded that fish respond to liberated CO_2 separately from the accompanying hydrogen ion concentration; they demonstrated a powerful hyperventilating response in Atlantic salmon *Salmo salar* at a reduced pH of 4·0. These observations and conclusions suggest that biomonitoring systems based on fish ventilation may be susceptible to a slight decrease in pH and the accompanying CO_2 produced if the dilution water is much harder than Lake Superior water.

Coughing responses of fish exposed to non-toxic environmental variables have received scant attention. Considerable work has been done on ventilatory frequency and stroke volume of fish exposed to low DO levels, but only a couple of observations exist on DO's effect on cough frequency. Hughes (1975) remarked that trout cough less frequently when subjected to hypoxia by lowering the PO_2 of inspired water, and Skidmore (1970) observed that the mean coughing frequency of rainbow trout was 1·1 coughs/100 opercular beats in high PO_2 but almost nil in low PO_2. The results here show that lowered DO has little, if any, effect on cough frequency.

There are conflicting observations concerning the effect of pH changes on cough frequency (Fromm, 1980), but these results (Tables 2 and 3) support those which showed little change at low pH and increased counts at high pH. Pegg & Jenkins (1976) reported bluegill coughing at pH 4·3 and suggested that coughing was one of many indicators of low pH-induced stress, but neither Lloyd & Jordan (1964) nor Neville (1979*a*,*b*) observed increased coughing in trout at pH concentrations around 4. Hargis (1976) observed significantly fewer coughs in rainbow trout at pH 6 and a greater number at pH 9 compared with rates at pH 7 and 8 and concluded that, unless the pH was controlled, or at least specified, the respiratory cough response was pH-dependent and not a reliable indicator of stress due to pollutants. However, Drummond *et al.* (1974) found that the cough frequency of brook trout controls was unaffected by changes in pH from 7·5 to 6·0 or 9·0. Neville (1979*b*) also pointed out that the trout used by Hargis had high cough rates (12 min^{-1}) in control water, suggesting the presence of a chemical irritant in the water. Lack of coughing in fish tested here was surprising since the lower pH threshold

for chronic effects such as tissue and cellular derangements of the gill was pH 5·2 for brook trout exposed 10 000 min (Daye & Garside, 1976). Coughing was the expected response to remove excess mucous accumulations from the gills. It is possible that a longer exposure period is required before coughing becomes symptomatic of gill damage due to acid waters.

Probably the most important physical factor regarding background cough frequency is suspended solids, due mainly to the ubiquitous presence of suspended matter in complex effluents and natural waters and the generally accepted assumption that coughing functions primarily to rid the gills of foreign debris that accumulates from even the cleanest of waters (Bijtel, 1949; Hughes, 1975). Satchell & Maddalena (1972) readily evoked parabranchial coughing in the Port Jackson shark *Heterodontus portusjacksoni* but did not elaborate on the concentration of suspended solids, nor did they make quantitative measurements of coughing. Green sunfish and largemouth bass *Micropterus salmoides* engaged in coughing when exposed to natural silt (Heimstra *et al.*, 1969). Sockeye salmon smolts *Oncorhynchus nerka* showed moderate cough reactions to 1% inner harbour sediment (Servizi *et al.*, 1969). And, finally, there was a definite relationship between the number of gill cleaning reflexes in fathead minnows *Pimephales promelas* and suspended conifer ground wood fibre concentration (MacLeod & Smith, 1966). From these observations, it seemed readily apparent that brook trout and bluegill sunfish would respond to clay suspensions, but their responses were highly variable. Brook trout did not increase coughing at all and bluegills showed significant, but only moderate, cough response in suspensions to around 300 NTU. It may be that the type, shape and size of suspended particles makes a great deal of difference as to whether or not a particulate suspension elicits coughing. Suspensions of wood pulp increased coughing in rainbow trout by eight- to ninefold, but coal dust with particle size that varied between 0·01 and 0·13 mm diameter was not so effective (Hughes, 1975). Coughing generally doubled in the coal dust but was not closely related to concentration. Kaolin suspensions evoked little increase in coughing even though they caused deposits on the gills, and Hughes (1975) felt that coughing was scarcely effective in dealing with small particulates. The clay particles in these tests were small, probably two microns or less in diameter, and it is possible that a larger clay particle is required to stimulate coughing in brook trout and elicit a stronger response from bluegill sunfish.

Another possibility to explain these and past variable results is that perhaps fish use different means for dealing with suspended matter, depending on the circumstances of exposure. Bluegill sunfish did not react at all with increased ventilatory frequency when exposed to the clay suspensions, but brook trout showed variable and, in one replicate at 231 NTU, significantly increased, ventilatory frequency. Horkel & Pearson (1976) found that green sunfish ventilatory frequency increased 50–70% in bentonite clay suspensions exceeding a turbidity of 898 Formazin Turbidity Units, and suggested that an increased ventilatory frequency helped prevent clogging of the gills with clay. It is possible that brook trout reacted similarly upon exposure to fine red clay particles, but that bluegill sunfish relied more on coughing manoeuvres to clear the gills. Regardless of what explanation best fits the data, these and past variable results encourage caution whenever applying fish ventilatory characteristics as a biomonitor where suspended solids loads are higher than normally encountered without first ascertaining what effect they may have as a separate variable.

The effect of dissolved solids on fish respiration is especially difficult to assess, partly because the literature is so sparse, but mainly because the results obtained here were variable Cairns *et al.* (1973) found no appreciable effects on the ventilatory responses of bluegill sunfish when calcium in the water was increased from 10 to 107 mg litre^{-1} (total hardness increased from 33 to 301 mg litre^{-1} as $CaCO_3$). This observation, plus the fact that a replicate group at 197 mg litre^{-1} (as $CaCO_3$) hardness showed no response, suggests that bluegill ventilatory and cough frequency responses at 190 mg litre^{-1} were an aberration, possibly due to the chance selection of particularly sensitive fish. Nevertheless, this variability must remain accountable when using a biomonitoring system that uses only four fish per concentration, and the no effect range was established accordingly.

Ventilatory and coughing responses such as those described herein were, for the most part, observed only in extreme concentration changes of these environmental variables. Such extremes are unlikely to occur in laboratory biomonitoring systems simply because most laboratories draw upon stable water supplies and, where they do not, these variables are easily controlled. In biomonitoring tests at this laboratory, for instance, dissolved oxygen has always been maintained above 90%, pH has varied by a few tenths of a unit at the most, dissolved solids of Lake Superior water have remained constant throughout and suspended solids

due to clay particles were easily filtered out of experimental waters. However, these idyllic conditions do not always exist for biomonitoring systems installed at the end of a final-treated effluent stream or near an outfall where they must draw upon upstream and downstream receiving waters. Under these circumstances, the latitude of ventilatory no-effect concentrations estimated for bluegill sunfish and brook trout exposed to these variables may easily be exceeded, especially for dissolved or suspended solids. Of the variables examined, these last two merit the most concern, mainly because so little research has been done regarding their effects on ventilatory and coughing responses and because the data used here for establishing their no-effect concentrations were limited and variable.

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